

Amendments to the Specification:

Replace paragraph [0045] with the following:

To evaluate the effect of liposome preparations comprised of the neutral lipopolymer on induction of complement activation, twelve liposome preparations and two micellar preparations were prepared, as described in Example 6. Table 3 in Example 6 details the lipid composition of the preparations. In brief and with reference to Table 4, the preparations included:

PREPARATION NOS. 1, 2, 3: two drug-loaded liposomes of identical lipid composition, differing only in the entrapped drug, doxorubicin (Doxil®) and cisplatin (preparation numbers 1 and 2) and a preparation of identical lipid composition but with no entrapped therapeutic agent, *i.e.*, placebo (preparation no. 3);

PREPARATION NO. 4: the effect of amount of PEG₂₀₀₀-DSPE on induction of complement activation was evaluated by comparing a preparation with 0.6 mole% PEG₂₀₀₀-DSPE with preparation no. 3 which was identical but for a higher (4.5 mole%) amount of PEG₂₀₀₀-DSPE;

PREPARATION NOS. 5, 6, 7: the effect of the negative charge of the PEG-DSPE was studied by comparing the preparation no. 3 (placebo to Doxil®) and the cisplatin liposome preparation nos. 1 and 2[[()]] with liposome preparations in which the negatively-charged PEG₂₀₀₀-DSPE was removed (preparation no. [[6]] 5) or replaced with two a neutral lipopolymer: PEG₂₀₀₀-DS (preparation no. [[7]] 6) and or PEG₂₀₀₀-DSG (preparation no. [[6]] 7; DSG=distearoyl glycerol; see Fig. 2A structure of mPEG-DSG);

PREPARATION NOS. 8, 9: the effect of the size of the PEG moiety on induction of complement activation was studied by comparing liposomes having negatively charged PEG-DSPE with different PEG molecular weights of 350 Daltons (preparation no. 8), 2000 Daltons (preparation no. 3), and 12,000 Daltons (preparation no. 9);

PREPARATION NO. 10: liposomes having a negative charge introduced through a liposome-forming phospholipid hydrogenated soy phosphatidyl glycerol (HSPG) were prepared for comparison with liposomes in which the negative charge was

introduced through the micelle-forming lipopolymer PEG₂₀₀₀-DSPE, which has a large headgroup (preparation no. 3);

PREPARATION NOS. 11, 12: as a liposome-positive control, liposomes of large particle size and composed of DMPC/chol/DMPG with cholesterol mole fractions of 50% (preparation no. 11) and 71% (preparation no. 12), as these preparations are highly potent in activating the complement system, including complement-dependent cardiopulmonary distress in pigs;

PREPARATION NOS. 13, 14: to determine whether PEG₂₀₀₀-DSPE without other lipids induces complement activation, micelles of PEG₂₀₀₀-DSPE (preparation no. 13) and PEG₂₀₀₀-DS (preparation no. 14) were prepared.

Replace paragraph [0049] with the following:

Fig. 6 shows the SC5b-9 induction, as a percent of the baseline SC5b-9 induction for cells incubated with phosphate buffered saline, for the indicated liposomal preparations. Liposome preparations 5 and 6 are neutral in charge (preparation no. 6 includes the neutral lipopolymer PEG-DS and preparation no. 5 is composed of the neutral lipids HSPC/Chol). These neutral preparations caused no measurable change in SC5b-9 formation. Preparation no. [[5]] 4 containing 0.6% PEG₂₀₀₀-DSPE also invoked little complement activation. However, all the other liposome preparations caused a significant elevation of SC5b-9 relative to the PBS control. The "Doxil® placebo" preparation no. 3 and the negatively charged HSPG-containing liposome preparation no. 10 caused moderate, approximately 2-fold rise in SC5b-9 formation, the Doxil® preparation no. 1 caused a very strong, 7-fold increase of SC5b-9. These data suggest that the negative electric charge and, particularly, doxorubicin in Doxil®, are contributing factors to complement activation. This finding was confirmed by the fact that liposome preparation no. 2, the cisplatin-loaded liposomes having the same lipid composition and size of the Doxil® liposome preparation no. 1 caused no or minor complement activation (data not shown). When HSPC was replaced by EPC as in preparation no. 7 (relative to preparation no. 6), a moderate but significant complement activation in 2/3 tested sera resulted.

Replace Table 5 with the following:

Table 5: Cardiopulmonary Response of Pigs to Different Liposomes

Preparation No.	Bolus Dose (nmole phospholipid/kg)				Reaction		
	5-30	30-150	150-1000	1000-10 ⁴	Frequency (%)	Grade ¹	n
1 – Doxil [®]	6	1			93	0	1/14
		1				II	1/14
		3				III	3/14
		3				IV	9/14
2 – cisplatin		2			0	0	2/2
3 – Placebo Doxil [®]	1		1		67	0	2/6
		1				II	1/6
		3				IV	3/6
4 – 0.6% PEG ₂₀₀₀ -PE			1		0	0	<u>1/1</u>
5 – HSPC/Chol	1		4	1	0	0	6/6
6 – PEG ₂₀₀₀ -DS				1	75	0	<u>1/4</u>
				3		I	3/4
7 - EPC/PEG-DSG	1				100	IV	1/1
8 – PEG ₃₅₀ -PE		2			33	0	2/3
		1				II	1/3
9 – PEG ₁₂₀₀₀ -PE		1			100	II	1/3
		2				III	2/3
10 – HSPG		3			100	II	3/5
			2			IV	2/5
11 – Low-chol	40				100	III	35/40
						IV	8/40
12 – High-chol	22				100	IV	22/22
13 – PEG-DSPE micelles	2				0	0	2/2
14 – PEG-DS micelles	2				0	0	2/2
15 – EPC/Chol/EPG	2				100	III	2/4
		2				IV	2/4

¹For definitions of grades see Example 7

²Lipid compositions of the preparations are given in Table 3 in Example 6.

Replace paragraph [0056] with the following:

Preparation nos. 16, 17, 18, and 19 all included HSPC and cholesterol, but differed in the lipopolymer. Preparation no. 16 included PEG-DSPE, similar to preparation no. 3 described above. Preparation no. 17 included PEG-DS and preparation no. 19 included HSPG.

Replace paragraph [0061] with the following:

A solution of mPEG₂₀₀₀ (20 g, 10 mmol) was azeotropically dried in toluene (50 mL, 120°C). After the temperature of the above solution reached 25 °C, it was treated with nitrophenyl chloroformate (3.015 g, 15 mmol) followed by TEA (2.01 mL, 15 mmol). This mixture was allowed to react for 1½ hr. The TEA-salt was filtered and the solvent removed to give crude mPEG₂₀₀₀-nitrophenylchloroformate, to which a solution of aminopropanediol (3 g, 30 mmol) in acetonitrile (50 mL) was added. This mixture was stirred overnight at room temperature. The insolubles were removed by filtration and the solvent was evaporated. The product was recrystallized twice from isopropanol. Yield: 13.7 g, 65%. ¹HNMR: (300 MHz, DMSO-D₆) δ 3.23 (s, OCH₃, 3H), 3.65 (s, PEG, 180H), 4.05 (t, urethane CH₂, 2H), 4.42 (t, 1°OH, 1H), 4.57 (d, 2° OH, 1H).

Replace paragraph [0062] with the following:

The product, mPEG₂₀₀₀ aminopropanediol (2.3 g, 1.08 mmol, 2.17 meq of OH), was dissolved in toluene (30 mL) and azeotropically dried, removing about 10 mL of the solution. The solution was allowed to cool to room temperature. Pyridine (4 mL, 20%) was added by pipette, followed by addition of stearoyl chloride (1 g, 4.3 mmol). Immediately a white precipitate was formed. The reaction mixture was refluxed overnight at 120°C and allowed to cool. When the temperature of the reaction flask reached about 40°C, the pyridine salt was filtered. The filtrate was evaporated. The product (PEG₂₀₀₀-DS) was purified by recrystallizing twice from isopropanol (2 × 30 mL) and dried *in vacuo* over P₂O₅.

Replace paragraph [0065] with the following:

In a 100 mL round bottom flask, ecosanoic acid (500 mg, 1.6 mmol) was dissolved in toluene (20 mL) and oxalyl chloride (147 µl, 1.68 mmol) was added by pipette. To the stirring reaction, 1% DMF was added. Upon addition of DMF, gas was released, as all contact with this gas should be avoided. After 10 minutes, the toluene was evaporated, and an additional 20 mL of toluene was added and evaporated to remove any excess of oxalyl chloride. The residue was redissolved in 10 mL of toluene. mPEG-aminopropanediol, prepared as described above, (1.19 g, 0.56 mmol)

was added to the solution, a reflux condenser was attached, and the mixture was refluxed overnight. Analysis by TLC (methanol and chloroform, 9:1) showed the reaction to be complete. After the reaction mixture cooled, the undissolved material was filtered, and the filtrate was taken to dryness. The product was purified by recrystallizing three time from isopropanol and dried *in vacuo* over P₂O₅. Yield: 1.0 [[mg]] g, 70%. ¹HNMR: (360 MHz, DMSO-D₆) δ 0.89 (t, CH₃, 6H), 1.26 (s, CH₂, 66 H of lipid), 1.50 (t, 2CH₂, 4H), 2.24 (t, CH₂CH₂ C=O, 4H), 3.23 (s, OCH₃, 3H), 3.50 (s, PEG, 180H), 4.00 (dd, CH₂ of APD, 1H), 4.05 (t, CH₂CH₂C+O, 4H), 3.23 (s, OCH₃, 3H, 3.50 (s, PEG, 180H), 4.00 (dd, CH₂ of APD, 1H), 4.05 (t, CH₂OC=O–N, 2H), 4.20 (dd, CH₂ of APD, 1H), 4.98 (m, CHOC(O), 1H), 7.34 (m, NH, 1H) ppm.

Replace paragraph [0068] with the following:

The salt was filtered from the reaction mixture, the solvent was removed by evaporation, and the solid was recrystallized twice from isopropyl alcohol and dried over P₂O₅. Yield: 9.2 g, 85%. ¹HNMR: (CDCl₃, 360 MHz) δ 1.25 (s, *t*-Bu, 9H), 2.82 (s, CH₂CH₂, 4H), 3.60 (s, PEG, 180 H), 4.45 (t, CH₂OCONH, 2H) ppm.

Replace paragraph [0106] with the following:

Four liposome (LUV) preparations were made. The liposome compositions and characterizations are set forth in Table 6. In preparing each of the formulations all lipid components of the formulation were dissolved in tertiary butanol. The clear solution was freeze-dried. The powder was hydrated in 10 mL hot (65°C) sterile pyrogen-free saline by vortexing for 1 minute at 70°C to form MLV. The MLV were downsized in two extrusion steps through polycarbonate filters of 0.4 and [[0.4]] 0.1 micron pore size 10 times through each using TEX 020 10 mL barrel extruded from Northern Lipids (previously Lipex), Vancouver, BC, Canada at 62°C. All steps of liposome preparation were done aseptically.